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1 **Estimates of heritability and genetic correlations for milk coagulation**
2 **properties and individual laboratory cheese yield in Sarda ewes**

3

4 A. Puledda¹, G. Gaspa¹, M.G. Manca¹, J. Serdino¹, P.P. Urgeghe¹, C. Dimauro¹, R.
5 Negrini^{2,3} and N.P.P. Macciotta¹

6

7 ¹ *Dipartimento di Agraria, Università degli Studi di Sassari, viale Italia 39, 07100 Sassari,*
8 *Italy*

9 ² *Associazione Italiana Allevatori, via G. Tomassetti 9, 00161 Roma, Italy*

10 ³ *Istituto di Zootecnica, Università Cattolica del Sacro Cuore, via Emilia Parmense 84,*
11 *29122 Piacenza, Italy*

12

13 Corresponding author: Giustino Gaspa. Email: gigaspa@uniss.it

14

15 **Short title:** Genetic parameters for clotting properties of milk

16

17 **Abstract**

18 Objective of this study was to estimate genetic parameters of milk coagulation properties
19 (**MCPs**) and individual laboratory cheese yield (**ILYC**) in a sample of 1 018 Sarda breed
20 ewes farmed in 47 flocks. Rennet coagulation time (**RCT**), curd firming time (**k₂₀**) and curd
21 firmness (**a₃₀**) were measured using Formagraph instrument (Foss, Hillerød, Denmark)
22 whereas ILYC were determined by a micro manufacturing protocol. About 10% of the milk
23 samples did not coagulate within 30 minutes and 13% had zero value for k₂₀. The average
24 ILCY was 36%. (Co)variance components of considered traits were estimated by fitting
25 both single- and multiple-trait animal models. Flock-test date explained from 13% to 28%
26 of the phenotypic variance for MCPs and 26% for ILCY, respectively. The largest value of
27 heritability was estimated for RCT (0.23±0.10) whereas it was about 0.15 for the other
28 traits. Negative genetic correlations between RCT and a₃₀ (−0.80±0.12), a₃₀ and k₂₀
29 (−0.91±0.09), and a₃₀ and ILCY (−0.67±0.08) were observed. Interesting genetic
30 correlations between MCPs and milk composition ($r_G > 0.40$) were estimated for pH, NaCl
31 and Casein. Results of the present study suggest to use only one out of three MCPs to
32 measure milk renneting ability, due to the highly genetic correlations among them.
33 Moreover negative correlations between ILCY and MCPs suggest a great care when using
34 these methods to estimate cheese yield from small milk samples.

35

36 **Keywords:** clotting properties, rennet, dairy sheep, genetic parameters, variance
37 components.

39 **Implication**

40 The estimation of genetic parameters is the first and essential step to select for
41 coagulation traits and cheese yield in dairy sheep. The aim of this paper is to fill a gap in
42 comparison to what happens in dairy cattle; indeed heritability estimates for clotting
43 properties are missing for small ruminants. Since the sheep milk is almost totally destined
44 for cheese making, the estimation of heritability of coagulation traits may enable future
45 scenarios of selection for such traits, with potential implications on selection schemes and
46 milk payment tables.

47 **Introduction**

48 The Italian dairy sheep stock consists of about 5.5 million ewes. The largest breed is the
49 Sarda with more than 3 million sheep (BDN, 2014). The Sarda breed accounts for about
50 43% of the national total ovine stock and about 4% of EU sheep stock (Eurostat, 2014).
51 The total milk production of the Sarda is about 300 000 t of milk per year (about 4% of total
52 world production, FAOSTAT, 2014). The breeding programme currently involves a
53 breeding nucleus of 212 941 milk recorded sheep farmed in 1 032 flocks, and the
54 commercial population (ICAR, 2014). Since the beginning of the program, the total milk
55 yield per lactation has been the main selective goal of Sarda sheep (Sanna *et al.*, 1997;
56 Carta *et al.*, 2009). Recording for fat and protein percentage on first lambing ewes started
57 in 1998. The milk is totally transformed in cheese with a production of 50-60 000 tons per
58 year of three Protected Designation Origin products, mostly destined for export (Furesi *et*
59 *al.*, 2013). Thus the milk cheese making ability could be a breeding goal of great interest
60 for this breed.

61 Pecorino Romano cheese yield could be predicted from bulk milk composition using
62 suitable equations (Pirisi *et al.*, 1994). However, predictions on individual milk are less
63 accurate due to the variability of milk solids, thus individual laboratory cheese yield (ILCY)
64 has been proposed as an indicator of potential cheese yield in individual ovine milk
65 samples (Othmane *et al.*, 2002). ILCY shows low heritability and a positive genetic
66 correlation with milk composition and negative with milk yield in Spanish Churra sheep
67 (Othmane *et al.*, 2002).

68 Other indicators of milk cheese making ability, extensively studied in cattle, are milk
69 coagulation properties (**MCPs**) (Aleandri *et al.*, 1989; Ikonen *et al.*, 1999; De Marchi *et al.*,

2008; Bonfatti *et al.*, 2014). They are usually defined by three parameters: rennet coagulation time (**RCT**, min), curd firming time (**k₂₀**, min) and curd firmness (**a₃₀**, mm), commonly measured by using either mechanical or optical devices (Bittante *et al.*, 2012). Several studies have reported that an appreciable proportion of the MCPs variation in cow milk is of additive genetic nature. A recent review by Bittante *et al.*, (2012) reported moderate values of heritability (about 0.26) for RCT and a₃₀, whilst few study report reliable estimates for k₂₀. In general, MCPs exhibit moderate to high genetic correlations with pH and somatic cell count, and very low to null with milk traits, respectively (Bittante *et al.*, 2012).

Few studies have been carried out on MCPs in small ruminants, especially in sheep. In particular effects of environmental factors, feeding, breed, parity and lactation stage on MCPs, milk composition and laboratory cheese yield, have been investigated (Jaramillo *et al.*, 2008; Bittante *et al.*, 2014; Pazzola *et al.*, 2014). In some researches, novel milk coagulation and syneresis parameters, estimated by a nonlinear modelling of the entire curd-firming process were used (Vacca *et al.*, 2015). Finally, Relationships between MCPs, sanitary status of the mammary gland were also investigated in sheep (Rovai *et al.*, 2015). Analysis of environmental factors affecting MCPs and ILCY and the estimation of their genetic parameters for MCPs and ILCY are essential steps for planning their improvement by means of selection. Aim of this study was to estimate heritability of MCPs, ILCY and their phenotypic and genetic correlations with milk yield and composition in Sarda dairy ewes.

91

92 **Material and methods**

93 *Animals, milk sample collection and laboratory analysis*

94 The study involved 1 018 Sarda ewes from 47 flocks located in the four historical
95 provinces of Sardinia. Pedigree and milk recording information were supplied by the
96 national association of small ruminant breeders (ASSONAPA, Rome, Italy). The pedigree
97 file included more than 1.8 million animal records. Dairy ewes were offspring of 499 rams;
98 other details about the structure of the population are given in Table 1. Individual milk
99 samples (100 ml) were collected in the mid-late lactation (from 45 to 249 days in milk,
100 average = 156 ± 37.4 days) from April to July 2014 by the provincial association of breeders
101 (APA). Milk samples were added with preservatives (bronopol, 62,5 μ l/100 ml). Analyses
102 were carried out within the 24 h after sampling and the milk samples were kept refrigerated
103 during transportation from the farms to the laboratory.

104 The milk samples were split into two subsamples of 50 ml each and analysed in order to
105 determine composition and cheese making attitude of milk, respectively. Standard milk
106 analysis were performed at the milk lab of the Regional Association of Animal Breeds of
107 Sardinia (ARA, Oristano, Italy). Milk composition was spectroscopically determined by
108 MilkoScanTM (Foss Electric, Denmark). Somatic cell count (SCC) was also determined
109 using the FossomaticTM (Foss Electric, Denmark). MCPs were measured by using a
110 Formagraph Instrument (Foss Electric A/S, Hillerød, Denmark). Briefly, 10 mL of each
111 individual sample were heated to 35° C before the addition of 200 μ L of rennet solution
112 (Hansen Naturen 215, with $80 \pm 5\%$ and chymosin $20 \pm 5\%$ pepsin, PacovisAmrein AG,
113 Bern, Switzerland) diluted to 0.8% in distilled water. This analysis ended within 30 min
114 after rennet addition and produced a lactodinamographic path as reported by Bittante *et*
115 *al.*, (2012). RCT is the time between rennet addition and the start of the milk coagulation,

116 k_{20} is the time at which the typical oscillation graph reaches the width of 20 mm, and a_{30} is
 117 the width of the graph at 30 min after rennet addition. ILCY was determined according to a
 118 modified method of Othmane *et al.*, (2002), further details of the methodology used are
 119 provided in Manca *et al.*, (2016). The predicted pecorino cheese yield (**PPCY**) was also
 120 calculated using the equation proposed by Pirisi *et al.*, (1994): $PPCY = 1.747 \times \text{protein\%} +$
 121 $1.272 \times \text{fat\%}$.

122

123 *Statistical Analysis*

124 Thirteen traits were analysed: RCT, k_{20} , a_{30} , ILCY, PPCY, milk yield (**MY**), fat percentage
 125 (**FP**), protein percentage (**PP**), casein percentage (**CSN**), conjugated linolenic acid
 126 percentage (**CLA**), pH, NaCl, Somatic cell score (**SCS**). Non-coagulating samples were
 127 eliminated, as well as the missing records for the other traits. Since the k_{20} parameter
 128 presented a skewed distribution, a log transformation was also applied on this trait.
 129 (Co)variance components were estimated by using Restricted Maximum Likelihood
 130 (**REML**) methodology implemented in VCE v. 6.0 software (Groeneveld *et al.*, 2010). Both
 131 a Single- (**ST**) and multiple-trait (**MT**) animal models were fitted. The raw data included 1
 132 018 animals with phenotypes. Ancestors were extracted from the pedigree file considering
 133 up to the fourth previous generation. A total of 5 234 animals were included in the
 134 numerator relationship matrix (**A**). ST and MT analysis were carried out on MCPs using
 135 the following linear mixed model
 136 $y_{ijklmno} = + + + + + + [1]$.

137 In the ST model, for each trait $y_{ijklmno}$ is a single measure for the i -th individual; μ is the
 138 overall mean; PAR is the fixed effect of j -th parity with 6 levels ($j = 1$ to 5 and $PAR \geq 6$); LM
 139 is the fixed effect of k -th lambing month with 6 levels ($k = \text{October to March}$); DIM is the
 140 fixed effect l -th class of days in milk with 6 levels ($l=1$: from 45-80d, $l=2$: 81-120d, $l=3$: 121-
 141 150d, $l=4$: 151-180d, $l=5$: 181-210, $l=6$: >210d); RP is the fixed effect of the m -th position
 142 of the milk samples in the rack of Formagraph, ($m = 1$ to 10); ftd is the cross-classified
 143 random effect of the n -th combination flock-test date ($n = 1$ to 70 levels) with
 144 $ftd_n \sim N(0, \mathbf{I}_{ftd}^2)$, where \mathbf{I} and σ_{ftd}^2 were the identity matrix and the variance associated to
 145 flock-test date, respectively; a_o is the random genetic effect for the o -th animal ($o = 1$ to 5
 146 234 levels) with $a_o \sim N(0, \sigma_a^2)$ and $e_{ijklmno}$ is the random residual term with
 147 $e_{ijklmno} \sim N(0, \sigma_e^2)$ where σ_a^2 and σ_e^2 are the additive genetic and residual variance,
 148 respectively. Genetic parameters of ILCY, milk yield and composition traits were estimated
 149 with a mixed linear model that had the same structure of eq. [1], but that did not included
 150 the effect of the rack position.

151 In the MT animal model \mathbf{y}_i represented the vector of dependent variables for the i -th
 152 individual, whereas the fixed and random effects were the same as eq. [1]. Two different
 153 MT analyses were carried out: i) a five-traits animal model, including the 3 MCPs, ILCY
 154 and PPCY, that was aimed at estimating genetic correlations among coagulation
 155 properties and cheese yields; ii) a series of bi-variate analyses for ILCY and each of the
 156 MCPs with all the remaining variables was performed to evaluate the genetic correlations
 157 among abovementioned properties and milk yield and composition. For both MT animal
 158 models the (co)variances for random effects were assumed to follow a multivariate normal

159 distribution and they were modelled as $[\mathbf{a}_1 \dots \mathbf{a}_n]' \sim N(0, \mathbf{A} \otimes \mathbf{G})$,
160 $[\mathbf{ftd}_1 \dots \mathbf{ftd}_n]' \sim N(0, \mathbf{I} \otimes \mathbf{F})$ and $[\mathbf{e}_1 \dots \mathbf{e}_n]' \sim N(0, \mathbf{I} \otimes \mathbf{R})$, where: \mathbf{A} and \mathbf{I} have previously
161 been defined; n was the number of traits analysed; \mathbf{G} , \mathbf{F} and \mathbf{R} were the n by n genetic
162 additive, flock-test-date and residual covariance matrices, respectively (the element $\sigma_{i,j(i=j)}^2$
163 in the diagonal are the variance and the off-diagonal $\sigma_{i,j(i \neq j)}$ covariance between trait i and
164 trait j). For each trait, both for ST and MT analyses, heritability was computed as
165
$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_{ftd}^2 + \sigma_e^2}$$
, for each analysed trait. Moreover, phenotypic and genetic correlations
166 among MCPs and all the other traits have been computed.

167

168 **Results**

169 *Descriptive statistics*

170 Fat, protein and casein percentages (Table 2) were similar to those observed in individual
171 data either in Sarda (Pazzola *et al.*, 2014; Manca *et al.*, 2016) and in other Italian breeds,
172 such as Valle del Belice (Cappio-Borlino *et al.*, 1997), Massese (Martini *et al.*, 2008) and
173 alpine breeds (Bittante *et al.*, 2014). Milk solid were lower than those reported for Spanish
174 breeds (Othmane *et al.*, 2002; Jaramillo *et al.*, 2008). The average values of the test-day
175 records for CLA (1.26 ± 0.57) was lower than values reported for Sarda and Massese
176 breeds (Nudda *et al.*, 2005; Martini *et al.*, 2008). The pH exhibited the smallest variability
177 whereas somatic cell count varied accordingly to the breed average (Pazzola *et al.*, 2014).

178 About 6% (n=64) of the samples did not coagulate within 30 min (**NC** samples
179 thereafter), and at the same time they did not present any values for a_{30} and k_{20} . The
180 samples with $RCT > 29$ min and $A_{30} < 1$ mm, about 9% (n=90) and 10% (n=101)
181 respectively, were discarded. Also 129 samples (about 13%) that did not reach a curd
182 firmness of 20 mm were excluded from the analysis (Figure 1). The percentage of NC
183 samples within each DIM class tended to increase along the lactation. However, when the
184 NC samples are referred to the total number of samples, largest values were observed in
185 the central DIM classes. The same trend is observed for the percentage of missing k_{20}
186 records (Figure 2).

187 The a_{30} and k_{20} parameters presented a more skewed distributions compared to RCT
188 and ILCY (Figure 1). The average values for RCT were lower than those reported by
189 Pazzola et al. (2014) and Vacca et al. (2015), whereas k_{20} and a_{30} varied accordingly to the
190 values reported in literature for dairy sheep (Table 2). Average ILYC measured in the
191 current study was $36.2 \pm 9.3\%$, whereas predicted cheese yield using the equation was
192 equal $17.3 \pm 2.4\%$ (Table 2).

193 A not negligible fraction of the phenotypic variance can be ascribed to the differences
194 between flocks. The flock-test date effect explained a quota of phenotypic variation
195 ranging from 13% to 33% for the three milk coagulation parameters and cheese yield
196 (Table 3). The largest percentage of variance explained by flock-test date was recorded for
197 MY (56%), followed by FP (50%), pH (30%), PP and CSN (20%). Moreover, for the last
198 two traits the flock variance matched those of MCPs.

199

200 *Heritability, phenotypic and genetic correlations of coagulation traits*

Heritability estimates (Table 3) were moderate for RCT ($h^2 = 0.23$) and lower for a_{30} and k_{20} ($h^2 = 0.15$). The estimate for ILCY ($h^2 = 0.16$) has doubled the values by Othmane *et al.*, (2002) heritability for PPCY was of the same magnitude of ILCY. Values for other milk traits ranged from 0.03 for SCS to 0.28 of NaCl content. Intermediate values were observed for MY (0.08), FP (0.10), PP (0.13) and CSN percentages (0.15) (Table 3).

Moderate to high phenotypic correlations were observed among coagulation traits (Table 4). The curd firmness at 30 min showed a negative correlation with RCT and k_{20} . Conversely, k_{20} was positively associated with RCT ($r_P = 0.84$). ILCY and PPCY presented moderate to low positive correlations with RCT- k_{20} and negative with a_{30} , respectively. Genetic correlations between MCPs were large and negative, those involving a_{30} , positive (between RCT and k_{20}) respectively. The largest value was for the correlation between k_{20} and a_{30} ($r_A = -0.91$). Genetic correlations for a_{30} and RCT are in the range of variability of MCPs as recently reviewed by Bittante *et al.*, (2012). Unexpected results were the negative genetic correlation between ILCY and a_{30} as well as the positive correlations between ILCY, RCT ($r_A = 0.55$) and k_{20} ($r_A = 0.64$). Conversely, PPCY was positively associated with a_{30} ($r_A = 0.22$) and negatively with k_{20} ($r_A = -0.19$) even if the magnitude of these estimates were lower than those involving ILCY and with larger standard errors. PPCY and ILCY were moderately associated ($r_A = 0.47$). Heritabilities of MCPs and ILCY estimated by the MT animal model were very close to those obtained with the ST model, with lower standard errors though (data not shown).

221

Phenotypic and genetic correlations between milk coagulation and milk quality traits

Phenotypic correlations among the three MCPs and milk traits were negligible apart from those involving SCS, pH and NaCl (Table 5). In particular, pH was negatively and moderately correlated with a_{30} and pH ($r_P=-0.42$) and positively and strongly with RCT, respectively. NaCl and SCS were moderately correlated with three MCPs. Moreover, RCT only was weakly associated to PP and CSN percentages. Phenotypic correlation of ILCY (PPCY) with fat and protein percentage were $r_P=0.46$ ($r_P=0.91$) and $r_P=0.37$ ($r_P=0.72$), respectively.

The majority of genetic correlations among MCPs and milk production and composition variables were close to zero (MY, FP with RCT) or presented very large standard errors (FP, PP, CS, SCS with a_{30}). Of interest are those between pH and RCT ($r_A= 0.68$) and pH and a_{30} ($r_A = -0.83$). RCT was also moderately correlated to casein ($r_A= 0.44$), but unexpectedly close to zero genetic association (with large standard errors though) were found among protein and casein and a_{30} . Moreover, both RCT and k_{20} showed a positive genetic association with NaCl, whereas, no reliable associations were found with functional compound like CLA. As expected, ILCY was positively correlated with milk composition (FP, PP and CSN) and negatively correlated with MY. High trivial genetic correlation were observed among fat, protein and casein and PPCY, and although of reduced magnitude when compared to ILCY, a negative correlation between PPCY and MY was observed.

Discussion

In general, studies on milk rheological properties are characterised by a relevant variability of results. Moreover, several variables affecting clotting properties have been identified so

246 far (Bittante *et al.*, 2012). In the present study, some milk samples did not coagulate within
247 the reference time of 30 minutes. The percentage of non-coagulating milks was larger than
248 in previous studies on Sarda and Alpine breeds (Bittante *et al.*, 2014; Pazzola *et al.*, 2014),
249 but smaller than the value (24%) observed on Sarda bulk milk (Giangolini *et al.*, 2004).
250 The result of the present study are similar to those observed in dairy cattle where the
251 proportion of samples that did not coagulate and those with missing k_{20} are on average
252 19% and 33% across studies, respectively (Bittante *et al.*, 2012).

253 According to some authors, the milk coagulation process should be faster in ovine than
254 bovine milk (Bittante *et al.*, 2012; Pazzola *et al.*, 2014). The average RCT found in the
255 present study does not confirm this hypothesis. It was twofold the values measured in
256 Sarda and Alpine (Pazzola *et al.*, 2014; Bittante *et al.*, 2014). On the other hand, it is in
257 agreement with results obtained in other studies on Sarda (Pirisi *et al.*, 2000; Mele *et al.*,
258 2006) Massese (Pugliese *et al.*, 2000; Martini *et al.*, 2008) and Spanish (Jaramillo *et al.*,
259 2008; Rovai *et al.*, 2015) sheep breeds. Average values and distributions of k_{20} and a_{30} are
260 in agreement with a previous report on Sarda ewes (Pazzola *et al.*, 2014).

261 Average ILYC measured in the current study was similar to those estimated by
262 Jaramillo *et al.*, (2008) but 10% higher than previous finding on Churra sheep (Othmane *et*
263 *al.*, 2002). Anyhow, the actual cheese yield is clearly overestimated by the use of ILCY.
264 Whereas the PPCY were in accordance to the average Pecorino Romano cheese yield
265 (Pirisi *et al.*, 2002) and it was moderately correlated with ILCY. The overestimation of
266 cheese yield could be ascribed to the method of micro-manufacturing used (Othmane *et*
267 *al.*, 2002; Bonfatti *et al.*, 2014) [see later in the discussion] .

268 Diifferently from what is observed in dairy cattle, the flock environment exerted a
269 significant role. Compared to previous works on Sarda (Pazzola *et al.*, 2014; Vacca *et al.*,
270 2015) the proportion of variance explained by flock-test day was similar for k_{20} , but slightly
271 lower for RCT and a_{30} . The fraction of variance explained by flock for MCPs was
272 dramatically higher in comparison with studies on cattle (Ikonen *et al.*, 2004; Tyrisev  *et*
273 *al.*, 2004; Vallas *et al.*, 2010), probably due to the peculiarities of sheep farming.

274

275 *Heritability of milk coagulation, composition and cheese yield traits.*

276 For some traits a significant quota of phenotypic variance was additive genetic, in other
277 cases the majority of the variation was of environmental nature. Estimates of heritability for
278 RCT were moderate and just in few case presented small standard errors. The
279 comparison can be made only with dairy cattle data due to the lack of information for
280 sheep in the literature. Values obtained in the present study confirmed reports for dairy
281 cattle (Ikonen *et al.*, 1999; Tyrisev  *et al.*, 2004; Cassandro *et al.*, 2008). However, RCT
282 heritability was below the findings of Ikonen *et al.*, (2004) and Vallas *et al.*, (2010). In the
283 case of a_{30} the heritability was of the same extent of other studies (Cassandro *et al.*, 2008;
284 Cecchinato *et al.*, 2011) but sensibly lower than Ikonen *et al.*, (1999; 2004) and Tyrisev  *et*
285 *al.*, (2004). The k_{20} parameter had a similar heritability of a_{30} but few reports have been
286 found on heritability of k_{20} in literature (Bittante *et al.*, 2012).

287 The heritability estimate of ILCY was double in magnitude compared to values reported
288 by Othmane *et al.*, (2002) whose estimates derived from a sample of similar size, even if
289 with ~7,500 test-day records of sheep milk over two generations. The cheese yield

equation-predicted on the basis of fat and protein percentage has an heritability of the same magnitude of ILCY. Heritabilities for milk composition traits were from low to intermediate. Values for fat and protein were markedly lower than those reported for Sarda sheep (Sanna *et al.*, 1997). The use of one test day per animal and the reduced sample size in comparison to other works, may at least partially justify these differences. However, values observed in the present study were close to those reported by Othmane *et al.*, (2002) and they were in the range of variability observed for dairy sheep (Oravcová *et al.*, 2005).

298

Phenotypic and genetic correlations between milk coagulation traits and cheese yield

The knowledge of genetic associations among coagulation, milk yield and quality traits is essential when exploring the possibility to select in favour of one of the MCPs traits. The overall phenotypic correlation pattern of MCPs confirmed what observed in Sarda and Churra breeds (Nudda *et al.*, 2001; Jaramillo *et al.*, 2008), whilst partially disagree with the results of Pazzola *et al.*, (2014). The latter authors found a low negative correlation between RCT and a_{30} (-0.15) indicating a substantial phenotypic independency between these two traits. In the present paper, moderate phenotypic and high negative genetic correlations were obtained between these two traits, respectively. This result is similar to previous reports in dairy cattle (Cassandro *et al.*, 2008, Ikonen *et al.*, 2004; Bittante *et al.*, 2012). Indeed, if milk takes less time to coagulate, then more time is available for the process of curd firming. Since the repeatability of RCT is quite higher (Bittante *et al.*, 2012), this means that also in sheep one measure of RCT is enough to predict both traits.

312 Furthermore, the strong negative phenotypic correlation between k_{20} and curd firmness at
313 30 min was expected, due to the positive association of k_{20} with RCT. Similar relationships
314 were previously found in Sarda sheep (Pazzola *et al.*, 2014). Additive genetic variance in
315 common between these two traits (k_{20} , a_{30}) have been scarcely investigated in dairy cattle,
316 due to the higher percentage of missing values for k_{20} parameter.

317 Negative correlation between ILCY and a_{30} (either phenotypic or genetic) and positive
318 correlations between ILCY and RCT- k_{20} were unexpected. Conversely, PPCY presented a
319 weak positive genetic association with a_{30} and negative with k_{20} . The possible explanation
320 for this correlation pattern can be formulated considering two conflicting aspects. The first,
321 is the interaction between predicted cheese yield and a_{30} as function of the fat percentage
322 (Aleandri *et al.*, 1989). These authors found that the predicted cheese yield was positively
323 associated with a_{30} with low fat milk, and negatively associated to a_{30} with high fat cow
324 milk. Hence, the high fat level of ovine milk, compared to cow milk, could partially explain
325 our results. A second issue is represented to the method used for measuring cheese yield.
326 Indeed micro-cheese factoring can produce biased estimation of actual cheese yield, due
327 to the small amount of milk used. This fact is also confirmed by the overestimation of
328 cheese yield, found also in other works (Othmane *et al.*, 2002; Jaramillo *et al.*, 2008).
329 Moreover, Bonfatti *et al.*, (2014) found that cow milk with short RCT and high a_{30} did not
330 exhibit higher cheese yield in model cheeses, being the cheese yield variation in their
331 experiment more likely associated to variation in milk fat and protein percentages. The
332 modest genetic correlation between the cheese yield predicted by regression (PPCY) and
333 a_{30} seem to suggest this second hypothesis, even if further investigations are needed to
334 clarify the relationship between ILCY and MCPs in sheep milk.

336 *Phenotypic and genetic correlation among milk coagulation, milk yield and composition*

337 The study of the genetic associations between MCPs and milk yield and chemical
 338 composition is crucial for evaluate proper selection strategies. The phenotypic correlations
 339 between RCT and protein and casein percentages found in the present study were in
 340 agreement with results on sheep (Jaramillo *et al.*, 2008; Nudda *et al.*, 2001) and in cattle
 341 (Bittante *et al.*, 2012). The worsening of the coagulation properties of sheep milk ($>RCT$
 342 and $<a_{30}$) with increased somatic cell count is documented in literature (Pirisi *et al.*, 2000;
 343 Nudda *et al.*, 2001; Raynal-Ljutovac *et al.*, 2007). An increased somatic cell count can be
 344 the also the result of intramammary inflammatory process (Rovai *et al.*, 2005). However in
 345 sheep, high somatic cell count in milk can be often unrelated with pathological conditions,
 346 differently from cow. Several factors (breed, parity, stage of lactation, type of birth, estrus,
 347 diurnal) affect SCC variation in sheep milk (Raynal-Ljutovac *et al.*, 2007). Increased RCT
 348 and k_{20} and reduced a_{30} with increasing pH were previously reported in sheep milk
 349 (Bencini *et al.*, 2002; Pirisi *et al.*, 2000). Finally, low to moderate phenotypic correlation
 350 were observed among individual cheese yield and milk traits. The highest association was
 351 between ILCY and fat percentage, and it was half of the correlation found by Jaramillo *et*
 352 *al.*, (2008) but agreed with the results of Othmane *et al.*, (2002).

353 Interesting genetic correlations have been estimated between pH, casein, NaCl and
 354 $RCT-a_{30}$. For the pH, similar values have been reported for dairy cattle (Ikonen *et al.*,
 355 2004; Cassandro *et al.*, 2008; Vallas *et al.*, 2010; Cecchinato *et al.*, 2011). Moderate
 356 correlations between RCT and casein percentage and no association among protein,

357 casein percentages and a_{30} were also reported by Ikonen *et al.*, (2004). On the other hand,
358 results of the present study were opposite to what found by some other authors
359 (Cassandro *et al.*, 2008; Cecchinato *et al.*, 2011). A suggestive negative association
360 between CLA with rennet properties might confirm what found by Bittante *et al.*, (2014) in
361 milk of sheep supplemented with rumen-protected conjugated fatty acid source.

362 Moderate to high positive genetic correlation were found between NaCl, a_{30} -k₂₀.
363 Conversely unreliable negative genetic correlation were found between SCS and RCT,
364 differently from what observed by other authors in cow milk (Ikonen *et al.*, 2004;
365 Cassandro *et al.*, 2008; Cecchinato *et al.*, 2011). A very high genetic correlation among
366 NaCl and SCS (0.98 ± 0.31) was found in the present study. This is an interesting results
367 because suggest the possibility to use indirect indicators of udder status (different from
368 SCS) linked to the rennet properties. As far as cheese yield concern, the positive genetic
369 correlation among milk composition and ILCY and negative with milk yield confirm what
370 found by Othmane *et al.*, (2002).

371

372 **Conclusions**

373 This study provided estimates of genetic parameters for milk coagulation properties of
374 sheep milk of Sarda Breed. From the selective point of view, a not negligible proportion of
375 phenotypic variance was additive genetic, and the heritability estimated for MCPs were in
376 agreement with those found in cow milk for MCPs. Genetic correlations found in the
377 present study suggest the chance to use only one of the rennet parameter, since they are
378 highly genetic correlated, however negative correlation between ILCY and favourable

379 rennet properties suggests to be careful in the use of this methods to predict cheese yield
380 from small milk samples.

381

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496

497 **Table 1** *Descriptive statistics for animals and flocks structure of Sarda population involved in this*
 498 *study.*

499

Items	n. ¹	average	sd	min	max
Flock size	-	95.5	57.1	13	233
Number of Lactation	1 016	4.0	2.3	1	12
Age (Month)	1 016	48.1	27.2	12	133
Ewes per ram	499	2.1	1.7	1	15
Rams per flock	47	16.9	5.8	6	33

500 ¹ For the number of lactation and age n. is the number of records; for the last two items, n. is the number of
 501 rams and flocks, respectively.
 502
 503

504 **Table 2** Number of records, overall mean, standard deviation, minimum (Min), Maximum (Max)
505 and coefficient of variation for sheep milk yield and composition, milk coagulation properties and
506 individual laboratory cheese yield.

Trait ¹	n. ²	Mean	SD	Min	Max	CV (%)
Milk yield and composition						
MY (L/day)	1 005	1.72	0.42	0.61	3.30	24.4
FP (g/100 mL)	998	6.06	1.35	2.55	12.00	22.3
PP (g/100 mL)	998	5.47	0.61	3.86	8.76	11.2
CSN (g/100 mL)	998	4.25	0.50	2.91	6.89	11.8
CLA (g/ 100g FAME)	908	1.26	0.57	0.00	3.04	45.2
SCS	994	4.68	2.33	0.06	11.13	49.8
pH (U)	1 002	6.58	0.14	5.65	7.36	2.1
NaCl (mg/100 mL)	998	146.88	45.16	64.30	551.70	30.7
Cheese-related traits						
RCT (min)	1 008	15.18	4.29	2.37	30.07	28.3
k ₂₀ (min)	879	1.75	0.74	0.50	7.00	42.3
logk ₂₀	879	1.99	0.16	1.48	2.62	8.1
a ₃₀ (mm)	990	52.63	16.08	0.98	107.80	30.6
ILCY (%w/v)	1 017	36.24	9.33	4.67	80.14	25.7
PPCY (%w/v)	998	17.28	2.43	11.71	29.55	14.1

507 ¹MY = test day milk yield; FP = fat; PP = protein; CSN = casein; CLA = Conjugated linoleic Acid, FAME=fatty
508 acid methyl esters; SCS = somatic cell score, $\log_2[(SCC_{\mu l^{-1}}/100)+3]$; RCT = rennet coagulation time; k₂₀ =
509 curd firming time; Logk₂₀ = \log_{10} of curd firming time in sec; a₃₀ = curd firmness; ILCY= individual laboratory
510 cheese yield; PPCY=Predicted Pecorino Cheese Yield,U=pH unit.

511 ² Number of samples used to compute descriptive statistics

512

Table 3 Estimates of genetic (σ_a^2) and environmental variance (σ_{ftd}^2 , σ_e^2), heritability (h^2) and percentage of variance explained by flock-test-day (r_{ftd}^2) and standard errors (SE) for sheep milk coagulation properties, individual cheese yield, milk production and composition traits.

Trait ¹	n. ²	σ_a^2	σ_{ftd}^2	σ_e^2	h^2 (SE)	r_{ftd}^2 (SE)
Cheese-related traits						
RCT (min)	908	5.83	3.32	16.17	0.23 (0.10)	0.13 (0.03)
$\log k_{20}$	879	0.004	0.006	0.016	0.15 (0.11)	0.23 (0.04)
a_{30} (mm)	907	27.74	54.92	110.49	0.14 (0.10)	0.28 (0.05)
ILCY (% w/v)	1 017	12.45	20.40	46.46	0.16 (0.09)	0.26 (0.05)
PPCY (% w/v)	998	0.66	1.55	2.46	0.14 (0.09)	0.33 (0.05)
Milk yield and Composition						
MY (L/day)	1 005	0.013	0.09	0.06	0.08 (0.05)	0.55 (0.04)
FP (g/100 mL)	998	0.16	0.76	0.71	0.10 (0.07)	0.47 (0.05)
PP (g/100 mL)	998	0.04	0.06	0.20	0.13 (0.10)	0.20 (0.04)
CSN (g/100 mL)	998	0.03	0.04	0.13	0.15 (0.11)	0.20 (0.04)
CLA (g/100g FAME)	908	0.02	0.14	0.12	0.09 (0.06)	0.50 (0.05)
SCS	994	0.16	0.72	4.18	0.03 (0.07)	0.14 (0.03)
pH (U)	1 002	0.003	0.006	0.01	0.16 (0.08)	0.30 (0.05)
NaCl (mg/100 mL)	998	543.1	289.1	1104.7	0.28 (0.13)	0.15 (0.03)

¹RCT = rennet coagulation time; $\log k_{20}$ = \log_{10} of curd firming rate; a_{30} = curd firmness; ILCY= individual laboratory cheese yield; PPCY=Predicted Pecorino Cheese Yield; MY = test day milk yield; FP = fat; PP = protein; CSN = casein; CLA = Conjugated linoleic Acid, FAME=fatty acid methyl esters; SCS = somatic cell score $\log_2(\text{SCC}_{\mu\text{L}^{-1}}/100+3)$, U=pH unit

²Number of samples used to estimate variance components.

522 **Table 4** Phenotypic correlation (below the diagonal) and genetic correlation (above the diagonal)
523 between sheep milk traits and milk coagulation properties estimated with a 5-trait animal model
524 (standard error in brackets).

Trait ¹	RCT	$\log k_{20}$	a_{30}	ILCY	PPCY
RCT (min)		0.84 _(0.09)	-0.80 _(0.12)	0.55 _(0.15)	0.08 _(0.21)
$\log k_{20}$ (min)	0.79		-0.91 _(0.09)	0.64 _(0.11)	-0.19 _(0.16)
a_{30} (mm)	-0.60	-0.76		-0.67 _(0.08)	0.22 _(0.17)
ILCY (% w/v)	0.41	0.32	-0.34		0.47 _(0.18)
PPCY (% w/v)	0.23	0.07	-0.13	0.51	

525 ¹RCT = rennet coagulation time; k_{20} = curd firming rate; a_{30} = curd firmness; ILCY= individual laboratory
526 cheese yield; PPCY=Predicted Pecorino Cheese Yield.
527

528 **Table 5** Phenotypic (r_P) and genetic correlation (r_G) among coagulation traits analyzed with bi-variate animal model in combination with sheep milk
529 yield and composition

Trait ¹	r_P					r_G				
	RCT	$\log k_{20}$	a_{30}	ILCY	PPCY	RCT	$\log k_{20}$	a_{30}	ILCY	PPCY
MY (L/day)	-0.09	0.07	-0.04	-0.09	-0.15	0.03 (0.39)	0.04 (0.48)	0.27 (0.46)	-0.88 (0.42)	-0.60 (0.46)
FP (g/100mL)	0.09	0.03	-0.12	0.46	0.91	-0.02 (0.38)	-0.34 (0.42)	0.32 (0.18)	0.45 (0.31)	0.93 (0.06)
PP (g/100mL)	0.30	-0.04	0.03	0.37	0.72	0.41 (0.32)	-0.42 (0.42)	0.09 (0.37)	0.75 (0.27)	0.85 (0.13)
CSN (g/100mL)	0.29	-0.06	0.05	0.38	0.73	0.44 (0.11)	-0.43 (0.45)	0.00 (0.48)	0.65 (0.27)	0.84 (0.13)
CLA (g/100g FAME)	-0.01	-0.05	0.14	-0.25	-0.37	-0.27 (0.38)	-0.46 (0.46)	0.27 (0.40)	-0.32 (0.34)	-0.33 (0.36)
SCS (U)	0.45	0.35	-0.30	0.35	0.29	-0.14 (0.92)	-0.72 (1.35)	0.11 (0.77)	0.58 (0.61)	0.29 (0.93)
pH (U)	0.70	0.55	-0.42	0.18	-0.28	0.68 (0.19)	0.44 (0.34)	-0.83 (0.21)	0.58 (0.42)	-0.21 (0.53)
NaCl (mg/100mL)	0.45	0.44	0.35	0.08	0.09	0.52 (0.27)	0.68 (0.32)	0.05 (0.51)	0.87 (0.65)	0.24 (0.51)

530 MY = test day milk yield; FP = test day fat percentage; PP = test day protein percentage; CSN = test day casein percentage; CLA = Conjugated linoleic Acid,
531 FAME=fatty acid methyl esters; SCS = somatic cell score $\log_2[(\text{SCC}\mu\text{l}^{-1}/100)+3]$, RCT = rennet coagulation time; k_{20} = curd firming rate; a_{30} = curd firmness;
532 ILCY= individual laboratory cheese yield; PPCY=Predicted Pecorino Cheese Yield.

533

List of figure captions

Figure 1 Frequency distribution of the three milk coagulation properties and the Individual laboratory cheese yield (ILCY) in Sarda sheep, for the raw data (before data editing). The very first (a_{30}) e the last two bars (RCT) represent those samples that have been discarded from the analysis.

Figure 2 Percentage of not coagulating (NC) or missing k_{20} samples (NoK20), percentage of not coagulating samples (NC_byDIM) and missing k_{20} (NoK20_byDIM) within each class of DIM.